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PRINCIPAL INVESTIGATOR: Vito Quaranta, M.D.

RECIPIENT ORGANIZATION: The Scripps Research Institute
La Jolla, CA 92037

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Table of Contents

Introduction: The subject of this research is breast cancer metastasis. Our long-term goal is to find cancer treatments based on targeting directly the metastasis process. An important element of innovation in our approach is that we visualize metastasis as a problem of breakdown in tissue organization. Consequently, for the purposes of cancer treatment, our target is the mammary gland as a tissue, rather than the individual cells. By taking a strictly reductionist approach, we are investigating the actual molecular mechanisms that keep breast epithelial cells segregated on the luminal side of the basal lamina. These epithelial cells are the ones from which invasive breast cancer arises. In our previous work, we identified a molecular mechanism (1, 2) that determines whether normal or cancer breast cells may or may not cross the basal lamina. This mechanism relies on the interaction of laminin-5, a major extracellular matrix molecule of basal lamina, with matrix metalloproteases and integrins. The specific challenge of this proposal is to determine how pervasive this mechanism is in regulating migratory versus stationary behavior of breast epithelial cells. If such mechanism is an important one, we will be one step away from entering a discovery phase for novel drugs or treatments that prevent or block breast cancer invasion.

Body:

AIM 1. To inhibit mammary epithelial cell motility in vitro and cell metastasis in vivo by blocking the migratory site of laminin-5 (Ln-5).

MIG-1 is an antibody to Ln-5 that blocks migration of cancer cells on Ln-5, after the latter has been cleaved by metalloproteases (MMPs). In this Aim, we originally proposed to map the cell migratory site defined by MIG1, to produce small molecules mimicking this site and test them in tumor invasion assays. By using western blotting, we mapped the MIG-1 epitope to the α3 chain of Ln-5. We then expressed fragments of the α3 subunit corresponding to its predicted folding domains, as GST fusion proteins. By further western blotting analyses of these α3 fusion fragments, we mapped the MIG-1 epitope to the G2 domain of α3 (Figure 1). This domain was then tested and shown to support adhesion of breast cancer cells. We will next test it in migration assays.
The MIG-1 epitope is now in the process of being mapped to a finer resolution, by site directed mutagenesis of the α3 G2 domain. Because MIG-1 is rat specific, we are using as a road map for mutagenesis the amino acid sequence differences between rat and human G2 (which are about 80% identical). We are confident that by this approach we should define a cell migration site no larger than approximately 5 kDa (as a reference, Ln-5 is 400 kDa). This size would be suitable to undertake the development of small molecular mimics that could inhibit cell migration.

AIM 2. To inhibit mammary epithelial cell motility in vitro and cell metastasis in vivo by inhibiting the cleavage of Ln-5 by MMP2.

Originally, we proposed to prepare Ln-5 fusion proteins containing the MMP2 cleavage

site, incubated them with MMP2, and analyze by western blotting and microsequencing whether they reproduced the original pattern of proteolysis as the original, intact Ln-5. We then proposed to test whether the fusion proteins will compete with Ln-5 as an alternative substrate for the enzymatic cleavage, and investigate whether they could block mammary epithelial cell migration in vivo.

We have changed our approach slightly and instead of producing fusion proteins in the GST system, we have produced the same Ln-5 fragments in the baculovirus expression system. The latter offers several advantages: 1. The desired fragment is not likened to a large fusion partner (GST) which could pose folding problems; 2. Glycosylation is more similar to physiological; 3. Production levels are 10 to 100 fold higher. The disadvantage of the baculovirus is that it is more labor intensive. However, we have already overcome that part, and have made two proteins containing the Ln-5 γ2 subunit domains that are cleaved by MMP2 (boundary between domain II and III). Furthermore, we recently identified another MMP cleavage site at the boundary of domain III and IV. We have produced a fragment including that site as well. We have now initiated testing to determine whether MMPs cleave these recombinant fragments.

Fig. 2

Aim 3. To produce monoclonal antibodies that react with MMP2-cleaved Ln-5 and not with intact Ln-5, and to use them in immunohistological assays for correlating the location of cleaved Ln-5 with breast cancer cell invasion sites.

We have not initiated this Aim as yet. We are however carrying out some groundwork. The limiting step for this Aim is the availability of purified Ln-5, both in cleaved and uncleaved form. Originally we proposed to use MCF-10 cells as a source of both, but have since ascertained that MCF-10 cells have a high background of spontaneously proteolyzed Ln-5. Therefore, we have screened several human cell lines that secrete Ln-5, and selected two that are high-producers. In one of them, Ln-5 is virtually entirely intact. We expect to initiate production of Ln-5 within 1-2 months, and then tackle antibody production.

Conclusions: We are very close to identifying the structure of the cell adhesion/migration site on Ln-5. This has been a long-standing question in the Ln-5 field, and therefore we expect our results to have significant impact. In spite of the fact that Ln-5 is clearly involved in metastasis, absence of structural details on its adhesion/migration domains has frustrated efforts to interfere with cancer invasion. By comparison, in the case of fibrinogen, another extracellular matrix molecule involved in blood clotting, knowledge of its adhesion site for platelets has led to the development of clotting pharmaceuticals that are already available to the public.

Similar considerations are applicable to our studies on the MMP2 cleavage site. MMP inhibitors are widely considered strong candidates as anti-metastasis drugs. In Aim 2, we have shown that a fragment of Ln-5 is cleaved by MMPs. This result was not obviously

predictable, and puts us in a position to eventually use the Ln-5 fragment as a basis for the design of MMP inhibitors.

References:

1. Giannelli, G., Falk-Marzillier, J., Schiraldi, O., Stetler-Stevenson, W.G., and Quaranta, V. Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. *Science* 277:225-228

2. Giannelli, G., Pozzi. A., Stetler-Stevenson, W.G., Gardner, H.A., and V. Quaranta. 1999. Expression of MMP2-cleaved laminin-5 in breast remodeling stimulated by sex steroids, *Am. J. Pathol.* 154:1193-1198.

Appendices:

Letter regarding unpublished data.
Figure 1.

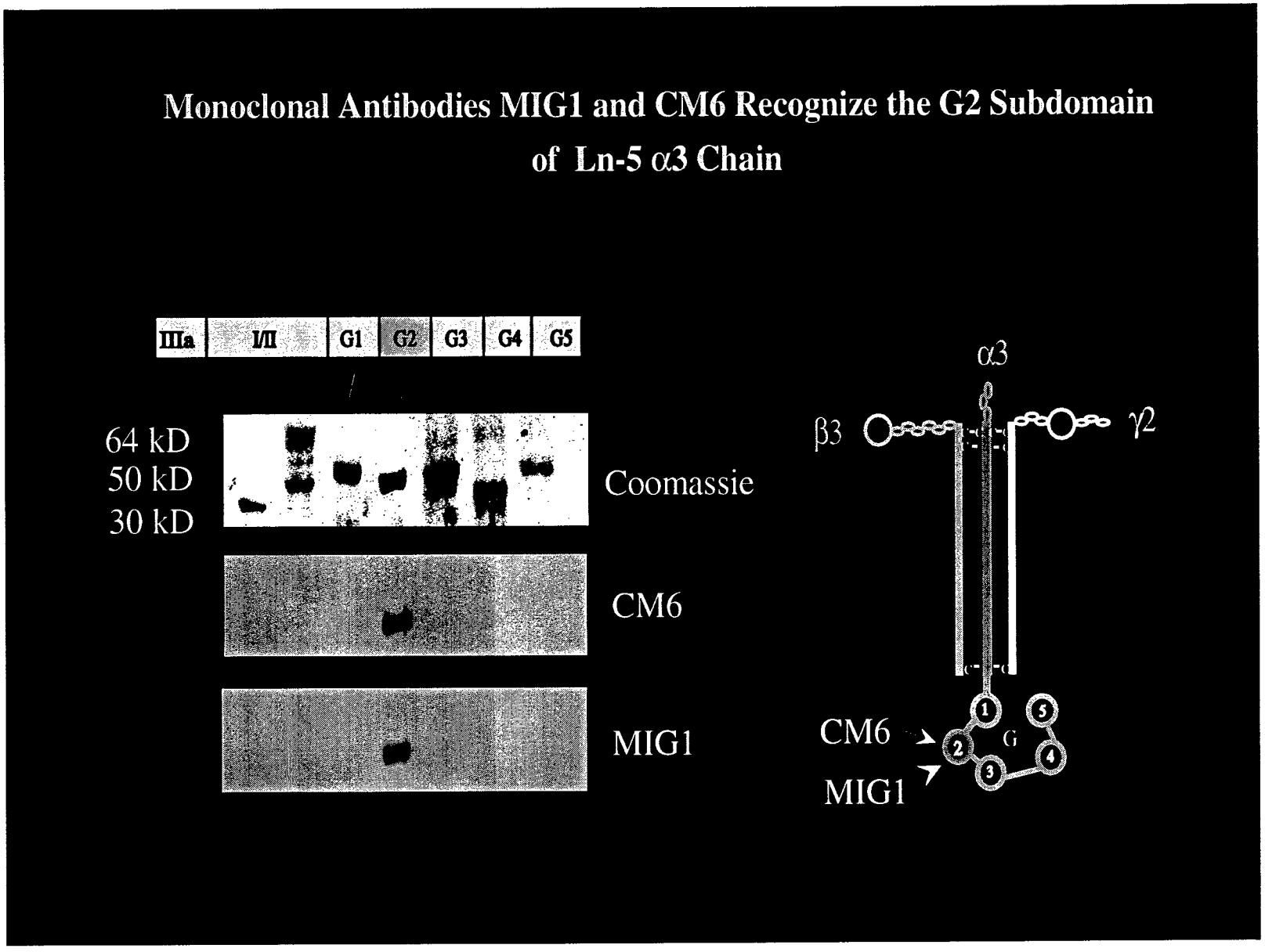
Monoclonal Antibodies MIG1 and CM6 Recognize the G2 Subdomain of Ln-5 α3 Chain
IIIa
I/II
G1
G2
G3
G4
G5
64 kD
50 kD
30 kD
Coomassie
CM6
MIG1
α3
β3
γ2
CM6
MIG1
G
1
2
3
4
5

Figure 1

Coomassie stain of purified domain III and III-V (Ln-5 γ2) from insect cells

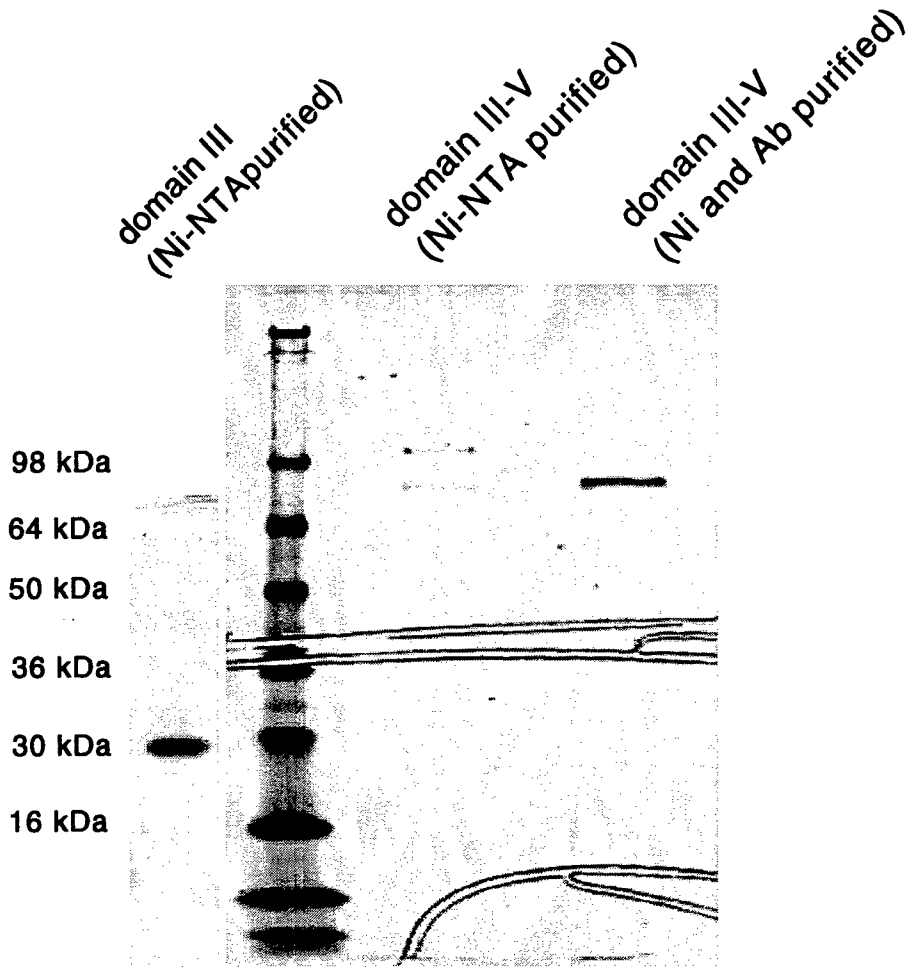

Figure 2

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FOR THE COMMANDER:

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management